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FINAL REPORT

HWRIC Project RR062

ECOTOXICOLOGICAL EVALUATION OF AREA 9 LANDFILL AT CRAB ORCHARD NATIONAL WILDLIFE REFUGE: BIOLOGICAL IMPACT AND RESIDUES

Submitted by:

Cooperative Wildlife Research Laboratory, SIUC

Presented to:

Hazardous Waste Research and Information Service

September, 1992

Prepared by:

Michael J. McKee, Assistant Professor
Cooperative Wildlife Research Lab.
Southern Illinois University
Carbondale, IL 62901

Approved by:

Alan Woolf, Director
Cooperative Wildlife Research Lab.
Southern Illinois University
Carbondale, IL 62901

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Michael J. McKee

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Contents

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I would like to thank Norrel Wallace, Project Manager for Crab Orchard National Wildlife Refuge, for his cooperation in this project. Mr. Wallace allowed access to all sites at the refuge and encouraged data collection to resolve issues regarding biological effects of chemical contaminants. Staff of the Illinois Environmental Protection Agency, especially Steve Davis, was very helpful in reviewing safety requirements for personnel accessing the Area 9 Landfill. A special thanks is extended to Steve and coworkers Scott Moyer and Ken Miller for collecting the soil core samples from the Area 9 Landfill. This was a particularly arduous task given an air temperature in excess of 95°F and donning of the Level C protection gear. The ice vests were a lifesaver! Leanne Moore, Contaminant Specialist at Crab Orchard National Wildlife Refuge, has provided constructive review of progress reports and sample collection methodologies. Rick Requarth has functioned as a research technician on the project. His commitment and thoroughness has made data collection a smooth and enjoyable process. Brett Ratcliff, University of Nebraska, generously donated his time to verify the identity of the coleoptera specimens collected in this investigation. I would like to thank Jennifer Burrow and Greg Simmons for their assistance in various phases of the project.

TABLES

FIGURES

ABSTRACT

Polychlorinated biphenyls (PCBs) and lead were investigated in soil and biota at Crab Orchard National Wildlife Refuge (CONWR) and related to biological effects. PCB levels in soils from Area 9 Landfill ranged from 25 to 7,300 mg PCB/kg soil (wet weight) and lead concentrations ranged from 40 to 5,000 mg lead/kg soil. PCBs were rapidly mobilized from the soil into the terrestrial food chain as evidenced by high residue levels in adult beetles, caged house crickets, and white-footed mice. Comparison of measured exposures for white-footed mice to the exposure predicted by the risk assessment of O'Brien and Gere (1988) indicates that initial estimates of high risk to mice and other small mammals occupying PCB contaminated sites at CONWR are accurate. However, our data indicates that inhalation exposure may be more important than indicated in O'Brien and Gere's exposure assessment. Bioaccumulation of lead was not observed in invertebrates, but was observed in white-footed mice.

Invertebrate abundance and biomarkers were evaluated for signs of toxic response to soil contaminants. The control site and the Area 9 Landfill did not differ in abundance of five common terrestrial invertebrate families. Similarly, family richness and diversity were not reduced at Area 9 Landfill compared to the control site. Biomarker analysis in honeybees and house crickets did not reveal significant adverse effects of PCBs or lead. The absence of detectable biological effects in invertebrates shows that these animals can tolerate relatively high environmental concentrations of these contaminants. The intensive use of Area 9 Landfill by invertebrates and their apparent tolerance of soil contminants eludes to the importance of chemical transfer to higher trophic levels, especially for PCBs.

CHAPTER 1
INTRODUCTION

Crab Orchard National Wildlife Refuge (CONWR) was created in 1946 and occupies 17,420 hectares in southern Illinois. Prior to 1946 the area was used as farmland and, during World War II, as an industrial area for the manufacture of munitions. Subsequent to 1946 other industries have utilized the area for manufacturing munitions, metal fabrication, plating, and the manufacture of printing inks and electrical components. Wastes from these industries were accumulated in several landfills on the Refuge.

A statewide survey of liver metal contamination in hunter harvested white-tailed deer indicated significant elevation of nickel and lead concentrations in animals collected at CONWR (Woolf et al., 1983). Additionally, routine monitoring of fish from Crab Orchard Lake identified elevated levels of mercury as early as 1977 (Hite and King, 1977). These early studies sparked more definitive investigations that identified several sources of contaminants at CONWR.

Preliminary assessments of the waste landfills indicated sufficient chemical hazard to warrant inclusion, in 1984, of several CONWR sites on the National Priorities List established by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (O'Brien and Gere, 1988). Subsequently, a Remedial Investigation initiated by the U.S. Fish and Wildlife Service and Sangamo Weston, Inc. was completed and released (O'Brien and Gere, 1988).

The remedial investigation was an extensive analysis of the type, magnitude and distribution of contamination at 19 sites at CONWR. Seven sites containing various amounts of polychlorinated biphenyls (PCBs), lead, cadmium, chromium, mercury, magnesium, and cyanide were selected for remediation. They are: Area 9 Landfill, Area 9 Building Complex, Job Corps Landfill, Old Refuge Shop, Fire Station Landfill, Water Tower Landfill, and Area 7 Plating Pond. Human health hazards are low principally because six of the seven sites (Figure 1) are closed to the public. Five of the 7 sites, however, were identified to pose moderate or high risks to wildlife (USEPA, 1988). These sites and their soil contaminant characteristics are summarized in Table I.

O'Brien and Gere (1988) established endangerment to wildlife at certain sites at CONWR using a risk assessment approach. The assessment of ecological risk can be separated into 3 components: exposure assessment, effects assessment and risk estimation (USEPA, 1989a; 1989b). Exposure to wildlife can be assessed by modelling uptake from environmental mediums, by measuring body burdens of chemicals in the field (Moriarty, 1988) or by measuring specific biomarkers which reflect exposure to a particular chemical (McCarthy and Shugart, 1990). Biological effects can be assessed by measuring levels of chemicals that produce changes in health and performance of individuals, populations or communities. Frequently used measures of biological effects include survival, growth, reproduction, population structure, community structure, and biomarkers of effects. Estimation of risk is calculated by comparing exposure and biological effects data.

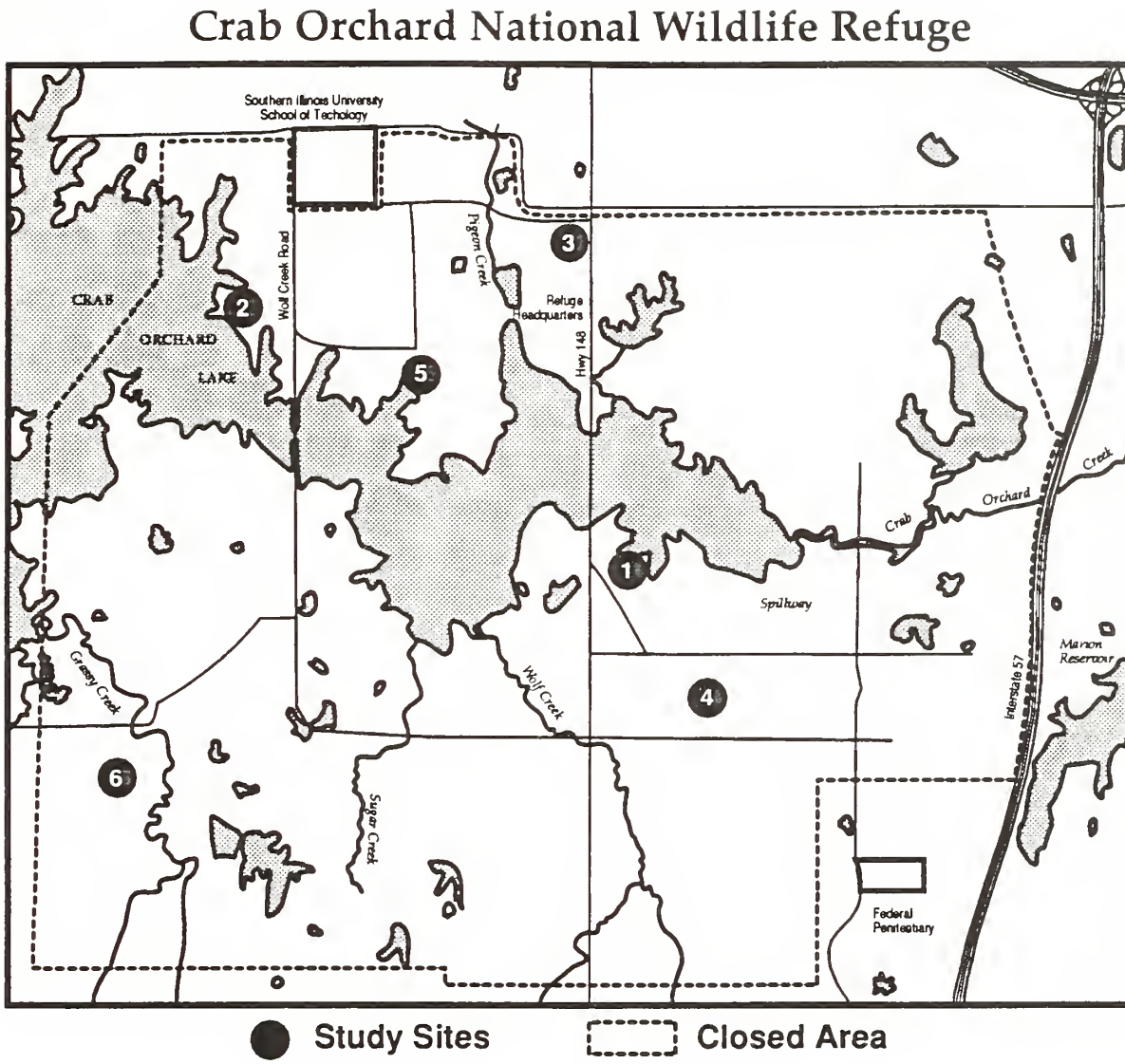

Figure 1. Location of study sites at Crab Orchard National Wildlife Refuge. 1 = Area 9 Landfill and Building complex (A9L), 2 = Job Corps Landfill (JCL), 3 = Old Refuge Shop (ORS), 4 = Water Tower Landfill (WTL), 5 = Olin site-control (OLS), and 6 = Area 13-control (A13).

Table 1. Contaminant characteristics of hazardous waste sites identified as posing moderate to high risk for wildlife.[a]

Site identification	Type of Contaminant	Environmental Medium	Maximum Contamination (mg/kg soil dry wt.)
Area 9 Landfill	PCBs	Surface soil	13,000 (wet wt.)
	lead	Surface soil	8,270
	mercury	Surface soil	35 ppb
Area 9 Buildings	PCBs	Surface soil	120,000 (wet wt.)
Job Corp Landfill	PCBs	Surface soil	69,042
	lead	Surface soil	17,410
	cadmium	Surface soil	57
Old Refuge Shop	cadmium	Stream sediments	780
	cyanide	Stream sediments	181
Water Tower Landfill	PCBs	Surface soil	8,900
	lead	Surface soil	4,300

[a] USEPA, 1988

Two aspects of the ecological risk assessment performed by O'Brien and Gere (1988) need refinement in order to better define the extent of wildlife endangerment at CONWR. First, calculation of risk was based on exposure levels for wildlife predicted by modelling uptake of chemicals from soil (O'Brien and Gere, 1988, Exhibit D). No attempts were made to verify these levels of exposure in the field. Secondly, biological effects data used in risk calculations were based on biological effects in laboratory animals and may have little relevance to natural systems. The objectives of this research parallel these 2 aspects of the O'Brien and Gere risk assessment and attempt to refine the data used to calculate risk to wildlife.

The principal and subordinate objectives of the research were as follows:

1. Determine body burdens of lead and polychlorinated biphenyls in certain animal species at CONWR as an estimate of exposure. Specific objectives were:

   A. to measure uptake from soil - beetles (Dyscinetus sp.), house crickets (Acheta domestica), and crayfish (Diogenes sp.) were selected as monitors because of their close association with soil;
   B. to measure uptake from air - The honeybee (Apis mellifera) was selected as an animal model to examine exposure via volatilization or particulate transfer from the soil; and

C. to measure consumer/predator exposure - Frogs (Rana pipiens sphenocephala) which are predaceous on many invertebrate species and white-footed mice (Peromyscus leucopus) which are omnivorous. These species were selected as representative of higher trophic levels in invertebrate food chains.

2. to determine biological effects in representative fauna. Specific objectives were:

A. to measure effects in insect community - richness and diversity of invertebrate fauna occupying Area 9 Landfill were monitored and compared to a control area;

B. to measure biochemical effects in insects - biomarkers in sentinel insect species were used as a measure of exposure and effects. Induction of ethoxyresorufin degradation was used as a biomarker of exposure (DiGiulio, 1989) and RNA concentration was used as a biomarker of effects (McKee and Knowles, 1989). Biomarkers were measured in honeybees and house crickets.

CHAPTER 2
DESCRIPTION OF SITES

CONWR is located at the southern terminus of the Illinoisan glaciation (Frye, 1965) which resulted in considerable topographic variation in the region. In general, areas north of the Refuge were glaciated producing flat relief suitable for farming. Areas south of the refuge tend to be hilly with rock outcroppings, much of which is unsuitable for agriculture. Several manmade lakes are present on the Refuge. Crab Orchard Lake, constructed in 1940, is the largest lake, accounting for about 16% of areal surface (2,800 hectares) at the Refuge.

Area 9 Landfill

Area 9 is a manufacturing site on the Refuge. It was leased to Sangamo Electric Co., Capacitor Division from 1946 to 1962 and is currently leased to Olin Corporation. Area 9 comprises an inactive landfill (Area 9 Landfill) (Figure 2, Appendix A) and the Area 9 Building Complex directly west of the landfill. Crab Orchard Lake is about 100 meters northeast of the Landfill and runoff can enter the lake through several intermittent creeks. Sangamo Electric manufactured various types of capacitors, utilizing aluminum, electrolytes, mica, silver, lead foil, and PCBs. Olin Corporation currently uses the buildings to manufacture explosives.

The landfill was closed in 1964 after nearly 2 decades as a repository for industrial waste (O'Brien and Gere, 1988). The limits of the landfill are discernible by changes in the topography and vegetation, revealing an area of approximately 1 hectare (2.5 acres) with an estimated fill thickness of 8 to 10 feet in the middle and 6 feet at the edges (O'Brien and Gere, 1988). The volume of the landfill is estimated to be from 16,000 to 35,000 cubic yards. About 80% of the landfill has vegetation cover dominated by Phragmites (reed grass) and Solidago (goldenrod). Materials visible on the surface appear to be electrical components consisting of small capacitors, capacitor parts, and a number of 3-inch steel cuplike pieces. A magnetometer survey suggests that the majority of wastes are buried along the eastern and northern edges of the landfill (Figure 2).

The Area 9 Building Complex is adjacent to the west border of Area 9 Landfill. The buildings were occupied by Sangamo Electric Co. between 1946 and 1962 and are currently occupied by Olin Corporation. Soil samples collected adjacent to several buildings in the complex contained PCB levels above 50 mg/kg wet weight. Some isolated samples collected along the sides of the access road to the Area 9 Landfill also contained elevated PCB concentrations (O'Brien and Gere, 1988).

Job Corp Landfill

The Job Corp Landfill (JCL), about 0.5 hectares in size, was active from 1951 to 1960 (O'Brien and Gere, 1988). The landfill is located adjacent to a one hectare pond constructed in the 1940s by the federal Job Corp (Figure 3, Appendix A). The landfill is almost completely covered with a variety of herbaceous and woody plants, however, widespread debris, such as bottles,

cans, mica flakes, small electrical contacts, and a few small capacitors can be observed on the surface of the ground.

The landfill was discovered during investigation of a Canada goose dieoff in 1985. About 30 geese carcasses in various stages of decomposition were found floating on the water or littering the shores. The Fish and Wildlife Service has completed chemical analyses of these carcasses and has not identified a potential causative agent. Conclusive evidence for the etiological agent in the dieoff was never obtained (O'Brien and Gere, 1988).

Old Refuge Shop

The Old Refuge Shop (ORS) site is located adjacent to the old Refuge Headquarters (Figure 4, Appendix A). According to the Refuge Manager, pine wood poles were treated in a fenced area of the Shop with pentachlorophenol wood preservative and shipped to various locations throughout the county (O'Brien and Gere, 1988). Contaminants were identified in a small drainage pool located immediately north of ORS, which drains through the woods to the northwest and, ultimately, to Crab Orchard Lake.

Water Tower Landfill

The Water Tower Landfill (Figure 5, Appendix A) was active in the 1940s and the 1960s (O'Brien and Gere, 1988). The landfill has received a variety of refuse including metal containers ranging in size from household dispensers to large drums. The site is nearly completely grown over by fescue and other grasses, greenbriar, and a variety of other shrubs.

Control Sites

Two control sites were established for this investigation. The Olin Site (OLS) was selected because it is similar in proximity, vegetation cover, and topography to Area 9 Landfill (Figures 2 and 6, Appendix A). In some cases, we needed to collect animals that occurred at A9L but not at OLS. In these cases we utilized a control site on the southwest part of the refuge (Figure 1). This location was farther removed from areas of known contamination than was OLS.

CHAPTER 3
METHODOLOGY

Summary of field residues collected

PCBs and metals were measured in soils, honeybees, crickets, beetles, crayfish, frogs, and small mammals collected at several of the hazardous waste sites at CONWR. These biomonitors were collected at Area 9 Landfill and Building Complex (A9L), Job Corps Landfill (JCL), Old Refuge Shop (ORS) and Water Tower Landfill (WTL) shown in Figures 2-5, respectively. Two control sites were utilized; the Olin Site (OLS) on the north side of the lake (Figure 1 and Figure 6) and Area 13 (A13) on the southwest side of the lake (Figure 1). A summary of the number and location of samples collected is shown in Table 2.

Table 2. Summary of residue samples collected at Crab Orchard National Wildlife Refuge.

	Number of Samples					
Type of sample	A9L	JCL	ORS	WTL	OLS/A13	Total samples per type
Soil	24	0	0	0	6	30
Honeybees	9	0	9	0	18	36
Beetles	6	0	0	0	5	11
Crickets	6	0	0	0	6	12
Crayfish	6	0	0	0	3	9
Frogs	10	0	0	0	2	12
White-footed mice	10	5	5	5	5	30
TOTAL						**140**

Collection of Field Residues

Soil Sampling - Three plots comprising 6 stations each were used to determine distribution of soil contaminants at Area 9 Landfill (Figure 2, Appendix A). Stations were arranged 10 meters apart in two rows each containing three stations. Location of the plots was based on proximity to previous soil core collection sites described by O'Brien and Gere (1988). A total of 18 soil samples were collected from the three plots at the Area 9 Landfill. Two container blanks were collected for quality control. A total of six soil samples (two randomly selected from each plot) were taken from 18 stations at the control site (Figure 6). Two container blanks were also collected at the control site.

Soil cores were drawn with hand held stainless steel core samplers 8 cm in diameter and 15 cm in length. Cores were collected by first clearing debris on the surface of the soil with a stainless steel knife, placing the

blades of the corer in contact with the soil and propelling the corer into the ground until the shaft of the corer was buried completely in the soil. The stainless steel core sampler and other equipment were decontaminated between each soil sample. Equipment was decontaminated by scrubbing in a tetrasodiumphosphate (TSP) solution, and rinsing sequentially with deionized (DI) water, acetone and DI water. Representatives from Illinois EPA supervised collection of those samples. Soil samples were stored at -20°C.

Honeybees - Hives of honeybees were established in May 1990 at four sites: Area 9 Landfill, Old refuge shop, and Olin site (control) (Figures 2, 4, and 6, respectively). Hives were initiated using honeybees purchased from Apis Company, 9449 Lackland, Overland, MO. Nine analytical samples of bees were collected in May 1990, at the time that bees were transferred from the parent colony in St. Louis, to determine background levels of metals and PCBs in the bees.

Hives at CONWR were sampled in June 1990, September 1990, and June 1991. About 100 g of bees (circa 1000 bees) were removed from the brood chamber at each sampling. Bees were collected by removing frames from the brood chamber and shaking adult bees into a funnel leading to a collection box. The boxes were transported to the laboratory and the bees immobilized by CO_2 exposure. Bees were removed from the box, collectively weighed, and transferred to a glass jar and stored at -20°C. Three replicate analysis of approximately 30 g each were performed for each hive at each sampling period.

Beetles - Portable ultraviolet light traps (Carolina Biological Supply Company) were used to sample beetles. In 1990, one light trap each was placed at Area 9 Landfill and Olin site (control) as shown in Figures 2 and 6, respectively. In 1991, traps were placed at Area 9 Landfill, Old refuge site, Job Corp Landfill, and Olin site (control) (Figures 2, 3, 4, and 6, respectively).

The 10 V portable light traps were actuated about 0.5 hrs after dusk by tripping a connector to a battery pack using mechanical timers. About 500 ml of deionized and distilled water was added to each trap. Beetles entering the trap were wetted by the water and unable to fly. Beetles were collected each morning using a stainless steel spoon previously rinsed with water, acetone, and water. Beetles were returned to the laboratory and separated by species. The sex of individuals was not determined. Samples were placed into 12 oz. mason jars pre-cleaned and pre-labeled using the sequence described previously. Aluminum foil was placed between the jar and lid. Samples were stored at -20°C.

Crickets - Adult house crickets (Acheta domestica) were selected as a sentinel species for assessing uptake of PCBs and metals in epigeic arthropod species. Initial attempts at in situ collection of sufficient insect biomass for chemical analysis were unsuccessful. Therefore, an in situ exposure cage was designed that would allow placement of house crickets in the field under semi-controlled conditions. Adult house crickets were purchased from Selph's Cricket Ranch in Mineral Wells, MI and housed in 40 l glass aquaria in the laboratory. For each field exposure, 50 crickets were randomly distributed among each of three replicates. Collectively, three groups were placed at the

Olin site (control) and three were placed at the Area 9 Landfill, for a total of 150 animals per site. Each group of 50 crickets was placed in a 25 cm^3 cage made of aluminum screen. The cage was placed in contact with the soil and covered with a larger cage (1 m X 1 m X 30 cm) to protect the crickets from predator harassment. Aluminum foil was used to shade the inner cages from the sun and the crickets were provided pelleted rat chow and tap water. Analytical samples were composed of both sexes in about equal proportions.

Three exposures were conducted; 1 for 3 days and 2 for 7 days. The 3 day exposure and one 7 day exposure were analyzed for lead and PCB uptake. Crickets from the other 7 day exposure were assessed for biological effects using the biomarkers EROD and RNA concentration (see biomarker section).

Crayfishes - Crayfish (Diogenes sp.) were collected on the north and east perimeter of Area 9 Landfill as indicated in Figure 2. Crayfish were collected by locating a soil chimney and excavating soil until the tunnel entrance was completely submerged in water. Crayfish could, in most cases, be lured from the tunnel by gently dangling an object (i.e. stick, finger, etc.) in the water for a few seconds. Sleuthing crayfish could be readily captured using a small minnow net. Individuals collected were placed in pre-cleaned containers and transported to the laboratory, weighed, and stored at -20°C. The majority of samples collected were male. Control animals were collected about 0.5 km southwest of Area 9 Landfill in an area not suspected of being contaminated.

Frogs - Southern leopard frogs (Rana pipiens sphenocephala) were collected near Area 9 Landfill as indicated in Figure 2. Ten pitfall traps, constructed of 10 cm (diameter) X 50 cm (length) sections of PVC pipe, were used to collect frogs between September 20 and September 28, 1989. A total of 19 frogs were trapped. Frogs were remove from traps and placed in pre-cleaned field sampling jars and returned to the laboratory. Animals were rendered unconscious by a blow to the head; rinsed in distilled water; weighed and stored in glass jars at -20°C. Sex of the frogs was not determined.

We were unable to locate southern leopard frogs at the Olin site. We did locate 4 southern leopard frogs in similar habitat near Carbondale. These animals were used to determine background levels and provide tissue for quality control.

White-footed mice - White-footed mice (Peromyscus leucopus) samples were collected at A9L, JCL, ORS, WTL and A13 (control site) in fall 1990. Samples were collected by distributing 60 Sherman live traps at 10 meter intervals along 2 transects (30/transect) at each of the following sites; A9L (Figure 2), JCL (Figure 3), ORS (Figure 4), WTL (Figure 5), and A13. Trapped mice were returned to the laboratory, weighed, sexed, aged, and sacrificed by cervical dislocation. Analytical samples were composed of pooled samples. Individuals were combined according to the location trapped; those individuals collected near the same location were pooled. In most cases this resulted in combined samples of a male and a female white-footed mice. Carcasses were stored at -20°C until analyzed for PCBs and metals.

Chemical Analysis

Analytical determinations of lead and PCBs were performed by the Animal Disease Laboratory, Centralia, Illinois. Each tissue or soil sample was homogenized using an Osterizer blender, Hobart mixer, or Robocoupe food processor, depending on the volume and consistency of the tissue. Lead, PCBs, moisture and lipid were determined on aliquots from the same sample homogenate. Quality assurance information comprising chain of custody forms and specific preparation protocols are available upon request from the Hazardous Waste Research and Information Center.

PCB analysis - Duplicate aliquots were weighed and extracted for 48 hours in a methylene chloride:cyclohexane (50:50) solution. Extracts were mixed with an equal weight of activated sodium sulfate and the supernatant was removed following centrifugation. The dried supernatant was subjected to clean-up using Gel Permeation Chromatography. Following the clean-up procedure, the samples were reduced in volume using rotary evaporation under vacuum and the concentrated extract was analyzed using gas chromatography.

Analysis was performed on a Hewlett-Packard 5890 Series II Gas Chromatograph fitted with an EC detector, auto injector and dual 30 m DB-5 column (0.25 μm). Data analysis was accomplished on a Hewlett-Packard Vectra QS 20 computer using Hewlett-Packard software. PCBs in tissue were quantified based on content of Aroclor 1254, which is the dominant component of the soil at CONWR (O'Brien and Gere, 1988). Quantification was accomplished by comparing 12 representative peaks of a standard Aroclor 1254 preparation to the same 12 peaks in extracted samples containing unknown quantities of PCBs. Final concentrations were expressed in μg Aroclor 1254/g wet weight of tissue. Quality control procedures included fortification analysis (recoveries > 95%), standard injection every third run and duplicate sample processing.

Metals analysis - Duplicate aliquots were weighed from the sample homogenate and analyzed for lead and, in samples from Old Refuge shop, cadmium. Dried samples were acid digested in nitric acid in a teflon container. Samples were diluted in de-ionized water and analyzed on a Perkin-Elmer 4000 Atomic Absorption Spectrophotometer with HGA-graphite furnace. Quality control procedures included standard injection every fifth run and duplicate sample processing.

Moisture and lipid analysis - Moisture for each homogenate was determined gravimetrically after drying samples at 100°C for 16 hours. Lipid was measured for each homogenate by determining specific gravity using a Foss-Let Fat Analyzer. Samples containing small amounts of lipid were analyzed gravimetrically using soxhlet extractions.

Biological Effects

Invertebrate and vegetation survey - Surface active invertebrates were sampled using pitfall traps as described by Southwood (1978). Pitfall traps were set into holes created by soil sampling. The traps were comprised of plastic cups with a smaller, inner cup and a plastic funnel. Traps were laid flush with the ground and the surrounding area was made as "natural" as possible. A 7 day sampling period was used in August 1989 and a 9 day sampling period was used in August 1990. Each sample was placed in a plastic, screw-top jar which had been labelled with site and station numbers. Cups were filled approximately 2 cm with 2% formalin to preserve the catch.

Invertebrate samples were cleared of dirt and debris using soil sieves (U.S.A. Standard Testing Sieves, Nos. 35 and 60) and rinsing with water. Specimens remaining on the screen were removed using watchmaker's forceps. This system recovered microarthropods, such as collembolans. Insects were identified to family using Borror et al. (1981). Shannon's diversity index (Ludwig and Reynolds, 1988), which combines information on richness and abundance of families, was calculated for each station using the following formula:

$$H' = - \Sigma \ (p_i \ \ln \ p_i)$$

where: n_i = the number of individuals in the ith family
N = the total number of individuals in all samples
p_i = the proportion of individuals of a given species (n_i/N)

Vegetation analysis of pitfall sample plots was conducted to facilitate comparisons between different plots. Six random samples of 1 square meter subplots were collected for each of the six plots. The three dominant species were identified, enumerated and recorded. In addition, the percent of ground visible in the square meter subplot was estimated and recorded.

Biomarker analysis - Ethoxyresorufin-O-deethylase activity and RNA concentration were determined in honeybees and house crickets collected from Area 9 Landfill and Olin sites (control). Five replicates were performed for each of the assays.

A 20% w/v crude homogenate (4 ml KCl-HEPES 1 g liver) was prepared by homogenizing whole honeybees, or midguts from house crickets in a Tekmar homogenizer for 1 minute. The homogenate was centrifuged at 10,000 g for 20 minutes at 4°C to remove nuclei and mitochondria. The post-mitochondrial supernatant was centrifuged at 105,000 g for 1 hour (Mazal, 1971). After centrifugation, the supernatant was decanted and the pellet (microsomes) was washed with 2 ml of cold KCl-HEPES. Samples were resedimented at 105,000 g for 1 hour and the supernatant decanted. For honeybees, the pellet was resuspended in 1 ml of KCl-HEPES buffer using a Potter-Elvehjm tissue grinder and assayed for enzyme activity. For crickets, the post-mitochondrial supernatant was used for enzyme activity measurement. The O-dealkylation of ethoxy-, and pentoxyresorufin by microsomal fractions was measured by a modification of the method by Lubet et al. (1985). Reactions were carried out in fluorometer cuvettes using a Hitachi F2000 fluorescence spectrophotometer. The 2 ml reaction mixture consisted of microsomal protein (20-40 μg), 12.5 μM NADPH and 0.05 M tris buffer pH 7.5 containing 0.025 M $MgCl_2$. The reaction

was started by adding enough substrate to make a final concentration of 5 μM in the mixture. The reaction rate was measured directly by reading the samples every 90 seconds and recording the fluorescence of the product, resorufin. Fluorometer settings for excitation and emission wavelengths were 530 nm and 585 nm respectively.

Total RNA was determined in whole homogenates of honeybees and in thoracic muscle tissue of crickets. A modification of the Schmidt-Thannhauser technique as described by McKee and Knowles (1986) was used. Briefly, whole homogenates were acidified with perchloric acid (PCA) to 0.2 N and centrifuged at 10,000 g for 20 minutes. The pellet was resuspended in 0.3 N sodium hydroxide and incubated at 37° C for 60 minutes. The incubate was adjusted to 0.2 N PCA and centrifuged at 10,000 g for 20 minutes. The supernatant was collected and analyzed for RNA content by measuring UV absorption at 260 nm. The pellet was resuspended in 0.6 N PCA and incubated for 15 minutes at 90° C. The sample was centrifuged at 10,000 g for 20 minutes. The supernatant was collected and analyzed by UV absorption at 268 nm. Protein was determined in the whole homogenate using the method of Lowry et al. (1951).

Quality Assurance and Quality Control

All research was conducted with authorization of the Project Manager at CONWR and the Illinois Environmental Protection Agency. The principal investigator has completed the 40-hour hazardous waste worker training course and was responsible for verifying that all workers were apprised of hazards that may exist and of their rights regarding exposure to hazardous waste substances. A copy of safety precautions for sample collection as specified by Illinois Environmental Protection Agency is available from Hazardous Waste Research and Information Center.

Standard operation procedures - Standard operation procedures (SOPs) were written for all methodologies or equipment that require continuity through time. SOPs were written, approved by the Director of CWRL, and retained in a reference binder for easy access by any employee. All dataforms used in the course of this investigation were approved by the Director and kept with its respective SOP.

Field sampling QA/QC - This research involved collection of field samples, transport to the author's laboratory, and, for residue samples, transport to the analytical laboratory. Several QA/QC procedures have been developed to facilitate this process. All field collection jars were cleaned and labeled in the laboratory prior to the field sampling trip. Field blanks were included for each field collection trip by including 1 extra jar subjected to identical laboratory processing. This "empty" sample was rinsed with solvent at the analytical laboratory and checked for analyte contamination using an appropriate technique.

A Chain of Custody (COC) form was designed to allow tracking of samples to the analytical laboratory. Each transfer of the samples required a signature, date, and time. All COC forms used in this study are available from Hazardous Waste and Information Center.

Analytical QA/QC- Quality control for analytical samples was assured by the use of fortified control samples to estimate the recovery of the compounds under experimental conditions. Injections of a range of standards that "bracket" the environmental samples were conducted each day and single standard injections were made every third sample.

Data Analyses

All statistical analyses were performed on Statistical Analysis Systems software (SAS Institute, 1985). Bioaccumulation was established by testing for significant elevation of residues between control and waste sites. T-test was used for comparisons involving 2 means. Comparison of more than 2 means was accomplished using ANOVA and the Least Significance Test. Significant biological effects were tested by comparing values from the control site to values from Area 9 Landfill using the t-test. The significance level was $P \leq 0.05$.

CHAPTER 4
RESULTS

Residue Analyses

Lead - Levels of lead in soil from the Olin site (control) averaged 19 ppm (μg/g wet weight) compared to a mean concentration of 900 ppm at Area 9 Landfill (Table 3). Coefficient of variation (CV) was 156% for lead samples collected at Area 9 Landfill compared to 8.6% for samples collected at the control area. Distribution statistics indicate that soil lead levels are high at Area 9 Landfill and that distribution of lead in soil at the landfill is not uniform.

Table 3. Lead concentration in soil collected from Olin site (control) and Area 9 Landfill (A9L) at Crab Orchard National Wildlife Refuge.

Site	n[a]	Moisture (%)	Lead, μg/g wet weight[b] Mean	Median	Range
Olin site (control)	6	11.4 (2.0)	18.7 (1.6)	19.0	16.4 to 20.5
Area 9 Landfill	18	14.0 (3.8)	900.0 (1408.0)	275.1	40.7 to 4956.5

[a] A sample (n) comprises at least 30 g of soil.
[b] Lead detection limit = 0.05 ppm

Lead does not readily move from highly contaminated soils into soil biota as evidenced by lead concentrations in beetles (Dyscinetus picipes), house crickets (Acheta domesticus), crayfish (Diogenes sp.), and southern leopard frogs (Rana pipiens sphenocephala) (Tables 4, 5, 6 and 6, respectively). Mean levels of lead observed in animals collected from the Area 9 Landfill did not differ from background levels of lead in surrounding areas. In general, variation patterns were similar between samples collected at Area 9 Landfill and other areas as indicated by similarities in the magnitude of the coefficients of variation.

Table 4. Lead concentrations in the beetle (Dyscinetus picipes) collected from the Olin site (control) and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Site	Date Collected	n[a]	Moisture[b] (%)	Lead[b] (μg/g wet weight)
Olin site (control)	June 1990	2	77.7	0.13
Area 9 Landfill	June 1990	3	69.9 (1.6)	0.14 (0.03)

[a] A sample (n) comprises at least 30 g of tissue. The number of individuals per sample is variable.
[b] Mean (standard deviation). Lead detection limit = 0.05 ppm

Table 5. Lead concentrations in caged house crickets (Acheta domesticus) exposed for 3 and 7 days to soil at the Olin site (control) and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Site	Days exposed	n[a]	Moisture[b] (%)	Lead[b] (μg/g wet weight)
Olin site (control)	3	3	62.0 (1.3)	0.57 (0.02)
	7	3	64.0 (4.6)	0.77 (0.51)
Area 9 Landfill	3	3	65.9 (5.5)	0.61 (0.13)
	7	3	71.1 (0.2)	0.59 (0.21)

[a] A sample (n) comprises at least 30 g of tissue.
[b] Mean (standard deviation). Lead detection limit = 0.05 ppm

Table 6. Lead concentrations in crayfish (Diogenes sp.) and southern leopard frogs (Rana pipiens sphenocephala) collected from the Olin site (control) and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Species	Site	n[a]	Moisture[b] (%)	Lead[b] (μg/g wet weight)
Crayfish	Olin site (control)	3	71.1 (1.5)	0.47 (0.14)
	Area 9 Landfill	6	73.3 (2.9)	0.38 (0.04)
Leopard frogs	Olin site (control)	4	80.6	0.21
	Area 9 Landfill	10	76.4 (1.4)	0.30 (0.09)

[a] A sample (n) comprises at least 30 g of tissue. The number of individuals per sample is variable.
[b] Mean (standard deviation). Lead detection limit = 0.05 ppm

Lead levels in honeybees (Apis mellifera) declined at all sites during the first 6 weeks of residence at CONWR (Table 7). However, after 3 months, lead levels were significantly elevated at the Old refuge site and a trend towards increased levels was observed at Area 9 Landfill. No differences were observed in lead concentrations among the 3 sites at the last sampling period (June 1991); and within a site, no differences were noted compared to initial honeybee levels (May 1990).

Table 7. Lead concentrations in honeybees (Apis mellifera) collected from the Olin site (control), Old refuge site and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Site	Date Collected	n[a]	Moisture[b] (%)	Lead[b] (µg/g wet weight)
Olin site (Control)	May 1990	9	77.1	0.42 (0.10)
	June 1990	3	69.2	0.22 (0.01) *
	September 1990	3	78.0 (0.3)	0.25 (0.06) *
	June 1991	3	71.2 (0.1)	0.49 (0.06)
Old refuge site	May 1990	9	77.1	0.42 (0.10)
	June 1990	3	73.2	0.21 (0.01) *
	September 1990	3	77.5 (0.7)	0.69 (0.24) *
	June 1991	3	69.7 (0.2)	0.69 (0.39) *
Area 9 Landfill	May 1990	9	77.1	0.42 (0.10)
	June 1990	3	70.2	0.23 (0.04) *
	September 1990	3	76.0 (0.4)	0.55 (0.09)
	June 1991	3	74.8 (0.2)	0.55 (0.03)

[a] A sample (n) comprises at least 30 g of tissue. The number of individuals per sample is variable.

[b] Mean (standard deviation). * denotes significant difference from May 1990 using one-way ANOVA and Least Significant Difference test. Lead detection limit = 0.05 ppm .

Lead was elevated in white-footed mice (Peromyscus leucopus) collected at the Water Tower Landfill as evidenced by a high mean lead level and a high coefficient of variation (132%) compared to other sites. Lead levels in the soil were high at the Water Tower Landfill (Table 8); however, not as high as at Area 9 Landfill or Job Corp Landfill. Mice collected on transects through these other sites were not significantly different from background (control).

Table 8. Lead concentration in white-footed mice (Peromyscus leucopus) collected at the Olin site (control), old refuge shop, water tower site, Job Corp landfill, and Area 9 Landfill at Crab Orchard National Wildlife Refuge during September 1990.

Site	n[a]	Moisture[b] (%)	Lead[b] (μg/g wet weight)
Olin site (control)	5	71.5 (1.3)	0.12 (0.07)
Old refuge shop	5	69.8 (0.7)	0.23 (0.18)
Water tower site	5	69.3 (7.6)	1.61 (2.12) *
Job Corp Landfill	5	72.9 (1.01)	0.56 (0.42)
Area 9 Landfill	10	68.9 (1.5)	0.30 (0.13)

[a] A sample (n) comprises at least 30 g of tissue.

[b] Mean (standard deviation). * denotes significant difference from Olin site using one-way ANOVA and Least Significant Difference test. Lead detection limit = 0.05 ppm.

PCBs- Soil concentrations of PCBs at Area 9 Landfill ranged from 25 to 7300 ppm among the 18 stations (Table 9). Distribution statistics reflected a skewed distribution similar to that observed for lead. Unlike lead, PCBs readily moved from the soil into soil associated invertebrates. Beetle adults (D. picipes) collected at Area 9 Landfill had significant residues of PCBs. The average weight of adult D. picipes is about 400 mg. Individual beetle residue levels based on this average weight would be about 2 μg/beetle for samples collected at Area 9 Landfill. Examination of chromatograms indicated a similar profile of congeners between soil samples and beetle samples, suggesting that the major route of exposure was direct soil contact of larvae. Beetles were collected from sites other than Olin site and Area 9 Landfill were sampled in 1991, however, insufficient biomass was available for replicate chemical analyses.

Table 9. Polychlorinated biphenyl concentration in soil collected from Olin site (control) and Area 9 Landfill (A9L) at Crab Orchard National Wildlife Refuge in August 1990.

Site	n[a]	Moisture (%)	μg PCBs/g wet weight[b] Mean	Median	Range
Olin site (control)	6	11.4 (2.0)	ND	ND	ND
Area 9 Landfill	18	14.0 (3.8)	1244 (1828)	320	25 to 7279

[a] A sample (n) comprises at least 30 g of soil.

[b] PCB detection limit = 0.005 ppm

Crayfish and southern leopard frogs accumulated similar levels of PCBs despite differences in collection location and habitat requirements (Table 12).

Table 12. Polychlorinated biphenyl (PCB) concentration in crayfish (Diogenes sp.) and southern leopard frogs (Rana pipiens sphenocephala) collected from the Olin site (control) and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Species	Site	n[a]	Lipid[b] (%)	PCBs[b] (μg/g wet weight)
Crayfish	Olin site (control)	3	0.31 (0.35)	ND
	Area 9 Landfill	6	0.45 (0.56)	0.73 (0.78)
Leopard frogs	Olin site (control)	2	1.07	ND
	Area 9 Landfill	10	1.14 (0.19)	0.81 (0.56)

[a] A sample (n) comprises at least 30 g of tissue. Moisture content of samples is given in Table 6.

[b] Mean (standard deviation). ND = not detectable (PCB detection limits were 0.005 ppm and 0.05 ppm for crayfish and frog, respectively).

Honeybees were not exposed to significant levels of PCBs as evidenced by the lack of body burdens in organisms collected from areas known to be contaminated by PCBs (Table 13). PCBs were detected in only one replicate of one sample from the Olin site in September 1990. The detection level was 0.01 ppm relative to the observed value of 0.09 ppm. Despite this one excursion above detection levels, evidence is strong in support of the observation that honeybees are not bioaccumulating significant amounts of PCBs at CONWR.

All sites known to be contaminated with PCBs in the soil yielded white-footed mice with significant body burdens of PCBs (Table 14). Body burden levels tended to reflect levels of soil contamination among the different sites with Area 9 Landfill being the highest.

Table 13. Polychlorinated biphenyl (PCB) concentration in honeybees (Apis mellifera) collected from the Olin site (control), Old refuge site and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Site	Date Collected	n[a]	Lipid[b] (%)	PCB[b] (μg/g wet weight)
Olin site (control)	May 1990	9	1.3 (0.3)	ND
	June 1990	3	1.2 (0.1)	ND
	September 1990	3	1.8 (0.2)	0.09
	June 1991	3	2.3 (0.0)	ND
Old refuge site	May 1990	9	1.3 (0.3)	ND
	June 1990	3	1.5 (0.5)	ND
	September 1990	3	1.9 (0.7)	ND
	June 1991	3	3.0 (0.1)	ND
Area 9 Landfill	May 1990	9	1.3 (0.3)	ND
	June 1990	3	1.1 (0.1)	ND
	September 1990	3	2.1 (0.2)	ND
	June 1991	3	3.1 (0.2)	ND

[a] A sample (n) comprises at least 30 g of tissue. Moisture content given in Table 7.

[b] Mean (standard deviation). ND = not detectable (PCB detection limit = 0.01 ppm).

Table 14. Polychlorinated biphenyl (PCB) concentration in white-footed mice (Peromyscus leucopus) collected at the Olin site (control), Old Refuge Shop, Water Tower Site, Job Corp Landfill, and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Site	n[a]	Lipid[b] (%)	PCBs[b] (μg/g wet weight)
Olin site (control)	5	1.28 (0.57)	ND
Old refuge shop	5	1.17 (0.45)	ND
Water tower site	5	1.14 (0.51)	0.08 (0.06)
Job Corp Landfill	5	1.18 (0.27)	1.75 (2.14)
Area 9 Landfill	10	1.53 (0.74)	11.16 (9.10)

[a] A sample (n) comprises at least 30 g of tissue. The number of individuals per sample is variable.

[b] Mean (standard deviation). ND = not detectable (PCB detection limit = 0.01 ppm).

Biological Effects Analysis

Invertebrate and vegetation survey - Abundance of epigeic invertebrates was investigated using pitfall traps placed at Olin site (control) and Area 9 Landfill. Pitfall traps were placed in the holes created by removal of the soil core samples; thus ensuring a reasonable estimate of potential exposure. Detailed information on families collected and relative abundance is given in Appendix A. Five representative families of terrestrial insects were analyzed for relative abundance (Table 15). No significant difference was noted between the control and waste site based on the abundance of these insects at the family level of classification.

Community parameters were calculated for both sites based on the family level classification (Table 16). The number of families (family richness) was significantly higher at Area 9 Landfill in 1989 compared to controls. In 1990, family diversity, which combines information on community richness and abundance, was significantly higher at Area 9 Landfill compared to the control site. These data suggest that invertebrates use Area 9 Landfill as much and perhaps more than the control site.

Evaluation of abundance of epigeic fauna can be greatly influenced by habitat. Dominant vegetation and percent ground cover were investigated as an estimate of differences between available habitat at the Olin site (control) and the Area 9 Landfill. All 3 plots at Area 9 Landfill were consistently dominated by goldenrod (Solidago sp.) and Reed grass (Phragmites sp.) based on abundance (Table 17). Similar consistencies were not seen at Olin site (Table 17).

Table 15. Abundance of five insect families captured in pitfall traps at Olin site (control) and Area 9 Landfill (A9L) at Crab Orchard National Wildlife Refuge.

Insect Family	Site[a]	Abundance[b] 1989	Abundance[b] 1990
Carabidae	Olin site	1.11 (0.83)	1.56 (2.18)
	A9L	1.06 (1.11)	4.11 (3.71)
Entomobryidae	Olin site	13.00 (12.88)	40.67 (26.63)
	A9L	19.06 (20.72)	17.28 (16.09)
Formicidae	Olin site	54.39 (66.66)	37.33 (21.87)
	A9L	27.33 (14.42)	26.83 (26.10)
Gryllidae	Olin site	1.05 (1.01)	1.22 (1.44)
	A9L	2.22 (1.80)	14.17 (13.26)
Staphylinidae	Olin site	2.44 (4.06)	1.06 (1.16)
	A9L	1.83 (2.55)	8.38 (12.13)

[a] Each site comprised 18 pitfall traps distributed among 3 plots.

[b] Mean and standard deviation of number captured/trap during sampling period. Sampling period was 7 days long in 1989 and 9 days long in 1990. Data were analyzed as a nested GLM (n=36). Asterisk denotes significantly different column means within family ($P \leq 0.05$).

Table 16. Comparison of family richness and diversity for insects at Olin site (control) and Area 9 Landfill (A9L) at Crab Orchard National Wildlife Refuge.

Community Parameter	Site	Sampling period[a] 1989	Sampling period[a] 1990
Richness	Olin site	18.00	28.67
	A9L	25.00 *	35.00
Diversity (H')	Olin site	1.06	1.71
	A9L	1.77	2.33 *

[a] Sampling period was 7 days in 1989 and 9 days in 1990. Mean of 3 plots for each site were compared with a T-Test (n=6). Asterisk denotes significantly different column means within a particular parameter ($P \leq 0.05$).

Table 17. Dominant vegetation types on Olin site (control) and Area 9 Landfill (A9L) at Crab Orchard National Wildlife Refuge.

Site	Plot #	Dominant Species	Plants/square meter[a]
Olin site	1	Goldenrod	24.0 (10.5)
		Foxtail	16.5 (3.5)
Olin site	2	Foxtail	50.0 (29.1)
		Goldenrod	17.5 (11.1)
Olin site	3	Foxtail	78.0 (9.6)
		Yellow foxtail	45.7 (9.5)
A9L	1	Goldenrod	30.2 (18.6)
		Reed	21.0 (11.6)
A9L	2	Goldenrod	37.8 (11.8)
		Reed	18.2 (20.0)
A9L	3	Goldenrod	55.7 (20.2)
		Reed	40.0 (33.9)

[a] Mean and standard deviation of 6 subsamples (1 square meter each).

although goldenrod was abundant on these plots. No significant differences were noted in the percent of ground covered by vegetation between the Olin site and Area 9 Landfill (Table 18).

Table 18. Vegetation cover at Olin site (control) and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Site	Plot #	Range of % cover [a]	Mean % Cover/[b] square meter
Olin site	1	70-85%	75.00 (6.33)
Olin site	2	80-95%	85.00 (7.07)
Olin site	3	75-99%	89.00 (9.49)
A9L	1	60-80%	71.67 (7.53)
A9L	2	30-90%	63.34 (24.22)
A9L	3	40-80%	66.00 (15.17)

[a] Maximum and minimum percent ground covered by vegetation for six subsamples (1 meter^2 each).

[b] Mean and Standard Deviation of 6 subsamples (1 square meter each).

Biomarker analysis - RNA concentration was not significantly reduced in insects collected from Area 9 Landfill compared to the control area (Table 19). These data suggest that no significant differences in growth rate are likely for these species under these conditions.

Ethoxyresorufin-O-deethylation is part of an enzyme detoxification complex that can be induced by exposure to PCBs. Consequently, measurement of this enzyme in sentinel organisms can be used to ascertain whether the organism has been exposed to PCBs. No significant differences were observed in honeybees or house crickets collected from Area 9 Landfill compared to the Olin site (Table 19), suggesting insufficient exposure to induce the enzyme or a lack of responsiveness of the enzyme in these species.

Table 19. RNA concentration and ethoxyresorufin-O-deethylase activity in honeybees and house crickets collected at Olin site (control) and Area 9 Landfill at Crab Orchard National Wildlife Refuge.

Species	Variable	n[a]	Olin site[b]	Area 9 Landfill[b]
Honeybee	RNA/DNA (μg/μg)	5	5.3 (1.4)	6.2 (1.7)
	EROD activity[c]	5	0.020 (0.003)	0.021 (0.001)
Cricket	RNA/DNA (μg/μg)	4	3.9 (1.0)	4.3 (1.6)
	EROD activity[c]	5	0.070 (0.021)	0.084 (0.029)

[a] An RNA sample is equivalent to 1 bee or 5 cricket thoraxes; an EROD sample comprises 20 g of honey bees or 5 cricket midguts.

[b] Mean and standard deviation.

[c] Ethoxyresorufin-O-deethylase activity is expressed as pmols/mg microsomal protein/minute.

CHAPTER 5
DISCUSSION

Lead

Lead does not biomagnify as it passes through terrestrial food chains. Although lead does not biomagnify, bioaccumulation is a common phenomena (Eisler, 1988). Bioaccumulation, as used in this discussion, is defined as uptake of environmental contaminants to levels greater than background. We observed bioaccumulation of lead in white-footed mice collected at Water Tower Landfill, but not at any of the other hazardous waste sites.

Another report has identified lead bioaccumulation in wild mammals at CONWR. Woolf et al. (1983), in a statewide survey of liver metal levels in hunter harvested white-tailed deer (Ordocoileus virginianus), found significant elevation of lead and nickel levels in samples collected at CONWR. White-footed mice have a small home range of about 0.1 hectare (Lackey et al., 1985) compared to several hectares for white-tailed deer. Consequently, exposure potential for mice occupying hazardous waste areas should be high compared to white-tailed deer, thus resulting in higher residues. However, lead bioaccumulation was not as extensive in white-footed mice as in white-tailed deer.

Several factors can influence bioaccumulation of lead in animals including food habits, soil contact, seasonal movements, and age. Age may be an important factor in explaining differences between lead accumulation in white-footed mice and white-tailed deer. White-footed mice in the wild rarely reach an age greater than 1 year (Lackey et al., 1985) whereas white-tailed deer commonly achieve an age of 3 to 4 years in the wild (Woolf, personal communication). Since lead concentration tend to increase with the age of the individual (Goyer, 1986), the short life span of white-footed mice compared to white-tailed deer may explain the lack of sensitivity of the mice as a lead biomonitor.

Another reason white-footed mice were not as sensitive to lead bioaccumulation as white-tailed deer, may be because whole body residue was measured instead of tissue specific concentration. Several authors have used whole body residues of white-footed mice to monitor lead contamination (Beyer et al. 1985; Clark, 1979). In these studies, whole body concentrations of lead in excess of 5 ppm wet weight were associated with contaminated areas. Although whole body residues of lead can be used to detect lead contamination, more sensitivity may be achieved by using tissue specific concentrations (Kisseberth et al., 1984; Scanlon, 1987; Welch and Dick, 1975). Woolf et al. (1983) investigated tissue specific metal concentration in white-tailed deer, whereas we investigated whole body burdens in white-footed mice. This may have contributed to our inability to detect widespread lead contamination in the small mammal population.

Data for the invertebrates collected at hazardous waste sites at CONWR suggest no tendency of lead to bioaccumulate. Lead levels in organisms likely to be exposed through soil, air, or food did not differ significantly from background levels in organisms from uncontaminated areas. Background levels

identified in biota in this study (i.e. 0.13 to 0.69 ppm wet weight or 0.43 to 3.1 ppm dry weight) are similar to levels identified in control animals from other studies (Eisler, 1988).

Polychlorinated biphenyls

Significant bioaccumulation of PCBs was observed in animals collected from areas with contaminated soil. Unlike lead, PCBs bioaccumulate in trophic levels and can biomagnify through food chains (Peakall, 1975; Risebrough et al., 1968). Uptake of toxicants by an organism is effected through ingestion, dermal, or inhalation exposures (Timbrell, 1989). Since route of exposure and, consequently, body burdens will vary according to habits of a particular species, it is important to consider differences in species habitats as well as their ecological roles.

Beetle grubs live in the soil and forage on plant roots for 2-3 years prior to emerging as adult beetles (Tashiro, 1987). Adults, especially males, are readily collected at UV light traps (Kard and Hain, 1990). D. picipes trapped at Area 9 Landfill had significant residues compared to beetles collected at other sites. Heida et al. (1985) previously reported on the use of beetles to monitor soil contamination. However, these authors investigated contaminant levels in grubs as opposed to adult beetles. Beetle grubs bioaccumulated tetrachlorodibenzodioxin (TCDD) yielding a bioaccumulation factor (BAF) of 3.2 (μg/g beetle:μg/g soil). For comparison, Lower et al. (1989) reported a bioconcentration factor of 0.13 for earthworms collected from soil contaminated with TCDD. A bioaccumulation factor cannot be calculated from this study since beetles were trapped away from the site of larval development. However, these data suggest that the beetle can be an effective monitor for soil contamination.

The use of light traps to collect beetle adults for the purpose of monitoring soil contamination, has not been previously reported. There are several advantages and disadvantages of this monitoring system relative to collecting specimens directly from the soil. The principal advantage is the ease of sampling. Light traps were automatically activated and adults were easily collected in the morning. Another advantage is personnel safety. Light traps can be set near the landfill, but not require personnel to enter restricted areas. Collecting beetle adults does not require disruption of soil as required by most other soil invertebrate collection schemes.

There are two major disadvantages to using beetle adults as biomonitors of soil contamination. First, bioaccumulation factors can not be calculated directly. Adults may fly some distance between daytime refuges and night-time mating rituals and feeding areas. An increased influx of adult beetles from areas remote to the contaminated site would "dilute" the level of contamination in pooled samples. Limited information is available on behavioral ecology of this group. Forbes (1907) indicated that migrations between daytime refuges and night-time feeding areas were generally as short as possible; adult beetles flying for the minimum time necessary. The problem of movement of adults can be partially remedied by assaying individuals as opposed to pooled samples. A second disadvantage is that it is impossible to separate exposure of the larva and adults. Adult beetles seek cover under

rocks and in vegetation during the day (Forbes, 1907). These habits could result in adult exposure to soil contaminants.

Frogs and crayfish were collected along the periphery of Area 9 Landfill and contained similar levels of PCBs. Both species occupy tunnels or burrows in the soil, but aside from this are quite different in their habits. For example, crayfish are scavengers, whereas, southern leopard frogs are primarily carnivores. Other investigation of PCB uptake by crayfish could not be found; however, Watson et al. (1985) investigated PCB uptake in green frogs (Rana clamitans melanota) and reported levels in tissue similar to that observed in this study.

Epigeic or soil dwelling fauna are an important component of terrestrial ecosystems. However, it is frequently difficult to obtain sufficient tissue biomass to provide meaningful contamination information. As a solution to this problem, we developed a test cage that would allow placement of invertebrates in situ. We selected the house cricket as a sentinel species because closely related species occupy sites at CONWR, and because crickets are readily available from several suppliers.

Cricket uptake of PCBs was rapid when placed at Area 9 Landfill. A particularly interesting observation in this study was that the crickets were not in direct contact with the soil. PCB uptake was effected through contact with airborne PCBs or by in situ contamination of soil or water present in the cricket cages. The importance of PCB volatilization as a means of exposure for epigeic fauna is suggested by the volatilization data collected by Lewis et al. (1985) for an uncontrolled landfill (Table 20). These data indicate that organisms living on the surface of contaminated soil could receive significant exposure to PCBs via the air. This hypothesis is supported by our findings using the caged crickets.

Table 20. Air concentration of polychlorinated biphenyls at uncontrolled landfill (Data from Lewis et al. 1985).

Distance Above Ground, cm	μg PCBs/m^3	
	July	October
2	580-1050	270-520
30	56-120	27-33
60	30-58	9-18
120	17-30	3-6
180	6-1	1-2

Honeybees have been proposed as a monitor of environmental contamination (Bromenshenk et al., 1986; Wallwork-Barber et al., 1982). Anderson and Wojtas (1986) found PCB residues of up to 56 ppm in dead bees collected from several

apiaries. They also detected PCBs in brood comb and honey. Honeybees in the present study did not accumulate PCBs. This was unusual considering the high degree of volatilization of PCBs from soil (Lewis et al., 1985) and adsorption of PCBs on plants (Buckley, 1987). The absence of PCB bioaccumulation in honeybees may be attributed to several factors. First, aerial transport may not be an important form of exposure. However, this is unlikely given the high degree of exposure observed in the crickets. Second, honeybees may have foraged away from the contaminated site thus avoiding exposure. Bees forage in a variable mosaic that can include an area 2 km in radius from the hive (Bromenshenk, In press). Although, this may contribute to the absence of residues in this study, honeybees were noted foraging in large numbers on goldenrod growing directly on the Area 9 Landfill. A third and most likely reason that bees did not reflect environmental PCB contamination was related to bee sampling methodology. Worker honeybees were collected from the brood chamber and these tend to be younger bees that have spent little time outside of the hive. Forager bees spend more time outside of the hive and have a higher potential for exposure than brood bees. Consequently, foragers are likely to have the highest body burdens of contaminants (Bromenshenk, In press).

Concentrations of PCBs in mice from this study contain the highest levels reported for white-footed mice collected at hazardous waste sites. Previous reports have established maximum body burdens of 3.0 ppm (Watson et al., 1985) and 4.2 ppm (Batty et al., 1990) compared to a maximum of 22.1 ppm in the present study (all concentration relative to wet weight). Soil contamination levels were not available from the other reports.

The exposure assessment performed by O'Brien and Gere (1988) estimated a mouse exposure rate of 45.6 mg kg^{-1} day^{-1}. Average concentration of PCBs in white-footed mice from Area 9 Landfill was 11.2 mg/kg. Based on the O'Brien and Gere exposure estimate, the average residue level could be achieved in less than one day of exposure (i.e. 11.2 mg/kg < 45.6 mg kg^{-1} day^{-1}). PCBs generally have high absorption rates (greater than 50%) and low excretion (less than 20%) (Safe, 1989). Using this toxicokinetic information and assuming several days at the exposure rate proposed by O'Brien and Gere (1988), it is easy to envisage body burdens in excess of those observed. However, this does not necessarily indicate that O'Brien and Gere's exposure rate estimate is unreasonable. For example, organisms may not be exposed continuously. Highly contaminated areas may make up only a small part of the home range for a particular animal, thus reducing overall exposure.

Interpretation of PCB residues is complicated by the nature of commercial Aroclor mixtures. Aroclor 1254 comprises about 30 of the 209 possible congeners of PCBs (Tanabe et al. 1987). Congener specific differences in toxicokinetics can significantly change the PCB profile within a particular organism (Hansen, 1987; Tanabe et al., 1987). We observed similar profiles between Aroclor 1254 and tissue profiles with the exception of white-footed mice. This species tended to retain about 5 major congeners similar to that observed by Anderson et al. (1991) for laboratory mice. However, congener specific analyses were not conducted in this study to identify which particular compounds were being selectively retained.

Biological effects

Community structure was not reduced by exposure to PCB contaminated soil as evidenced by family level classification of invertebrates collected in pitfall traps at the Olin site (control) and Area 9 Landfill. Moreover, increases were observed in family richness (1989) and family diversity (1990) at the Area 9 Landfill. Interpretation of these data, as well as data from other invertebrate surveys, is difficult because of problems associated with matching habitats at control and test sites (Ludwig, 1991). We identified differences in dominant vegetation among plots within a site and between sites. Minor shifts in vegetation composition can have major effects on the invertebrate community.

Results from the invertebrate survey conclusively show that Area 9 Landfill receives extensive use by invertebrates despite high levels of lead and PCB contamination in soil. High usage patterns by invertebrates coupled with rapid uptake as evidenced by the in situ cricket assay demonstrates the importance of this route in contamination of terrestrial food chains.

The absence of biological effects on community structure and biomarkers in invertebrates may reflect insensitivity rather than the absence of toxic effects. Sensitivity of community structure parameters relative to responses of individual organisms is not clear (Kooijman et al., 1987). It is possible that individual organisms are affected, but community richness and diversity remain unchanged. Likewise, response of insect RNA concentration and EROD to PCB and lead in soil has not been investigated. Recent investigations by Burrow and McKee (unpublished data) have found that mortality occurs in house crickets exposed to soil concentrations of Aroclor 1254 similar to that which occurs at Area 9 Landfill. Biomarker investigations in these laboratory studies will provide background information regarding the sensitivity of these tools for in situ assessments.

More information is available regarding the potential toxic effects of lead and PCBs in vertebrates. In our study, lead did not accumulate in white-footed mice to levels known to produce toxicity (Kisseberth et al., 1984). Therefore, we would not expect to see changes in biomarkers or other measures of individual performance. PCB levels, however, were much higher than lead. Sanders and Kirkpatrick (1975) identified food concentrations of PCBs that would cause toxicity in white-footed mice, however, no body burden information was reported. Simmons and McKee (unpublished data) have identified in laboratory studies that hepatotoxicity occurs in white-footed mice with body burdens of about 2 μg PCBs/g wet weight. Body burdens of PCBs in field collected white-footed mice frequently exceed 2 μg PCBs/g tissue suggesting that toxic effects are likely in the field.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Polychlorinated biphenyls (PCBs) are rapidly mobilized from the soil into the terrestrial food chain. Uptake of PCBs from the soil may be by direct absorption in the case of subsoil dwelling organisms, such as beetle grubs, or may be by aerial transport as evidenced by the in situ cricket bioassay. Based on invertebrate contaminant levels, food-chain transfer is at least partially responsible for the high levels of PCBs identified in white-footed mice. Comparison of white-footed mice body burdens to the exposure rate estimated by O'Brien and Gere (1988) indicate that initial estimates of high risk to mice and other small mammals occupying PCB contaminated sites at CONWR are accurate. However, our evidence indicates that airborne PCB exposure may be more important than indicated in O'Brien and Gere's exposure assessment.

Lead did not show a strong propensity to bioaccumulate in invertebrates and only one vertebrate sample (white-footed mice) was found to have elevated levels of lead. The absence of extensive bioaccumulation of lead in these species may reflect their relatively short life span rather than the absence of lead movement through the wildlife communities.

Biological effects were not observed in the insect fauna as evidenced by community analysis at the family level of classification and biomarker analyses. However, based on the levels of contaminants found in biota at CONWR, it is likely that toxic effects are occurring in some environmental receptors in or around Area 9 Landfill. Although, detection of toxic effects in insects may be difficult, other organisms that forage on contaminated insects may demonstrate more pronounced toxic effects, especially for PCBs.

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Table 2. Abundance of insect families found at the hazardous waste site and control site during the 1990 season.

Order	Family	Number Observed (total)[a] HWS	Olin
Collembola	Entomobryidae	311	732
	Hypogastruridae	06	416
	Sminthuridae	55	14
Microcoryphia	Meinertellidae	31	00
Orthoptera	Acrididae	53	01
	Gryllacrididae	05	09
	Gryllidae	255	22
	Tettigoniidae	03	00
Blattaria	Blattidae	24	04
Psocoptera	Ectopsocidae	03	00
Hemiptera	Aradidae	00	01
	Corimelaenidae	00	02
	Lygaeidae	36	42
	Miridae	00	05
	Nabidae	00	01
	Rhopalidae	06	00
	Reduviidae	01	00
	Scutelleridae	01	00
	Tingidae	00	07
Homoptera	Aphididae	00	16
	Cercopidae	03	00
	Cicadellidae	28	18
	Flatidae	02	00
	Membracidae	01	00
Thysanoptera	Thripidae	05	00
Coleoptera	Brunchidae	01	00
	Byrrhidae	04	03
	Cantheridae	00	04
	Carabidae	74	28
	Chrysomelidae	35	40
	Coccinelidae	00	01
	Curculionidae	05	02
	Dermestidae	05	00
	Elateridae	35	03
	Meloidae	01	00
	Mordellidae	12	01
	Ptilodactylidae	02	03
	Scarabaeidae	00	03
	Staphylinidae	149	19
	Tenebrionidae	02	00

Table 2 (continued). Abundance of insect families found at the hazardous waste site and control site during the 1990 season.

Order	Family	Number Observed (total)[a] HWS	Olin
Diptera	Agromyzidae	01	00
	Cecidomyiidae	04	03
	Chironomidae	09	01
	Micropezidae	01	00
	Muscidae	03	02
	Phoridae	14	10
	Sciaridae	04	04
	Sphaeroceridae	02	00
	Tachinidae	01	00
	Tephritidae	15	56
	Tipulidae	01	00
Lepidoptera	Hesperiidae	01	00
	Lycaenidae	00	01
	Noctuidae	00	01
Hymenoptera	Anthophoridae	01	00
	Apidae	01	00
	Bethylidae	00	01
	Braconidae	00	02
	Ceraphronidae	01	00
	Colletidae	03	01
	Eucoilidae	01	00
	Eurytomidae	00	01
	Formicidae	483	627
	Halictidae	11	04
	Megachilidae	02	00
	Perilampidae	01	09
	Pompilidae	00	01
	Pteromalidae	02	00
	Sphecidae	06	00
	Tiphiidae	00	01
	Torymidae	07	02
	Vespidae	01	00

[a] Total catch for 3 plots at each site over a 9 day sampling period.

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APPENDIX A

Figures of study sites at Crab Orchard National Wildlife Refuge

AREA 9 LANDFILL SITE

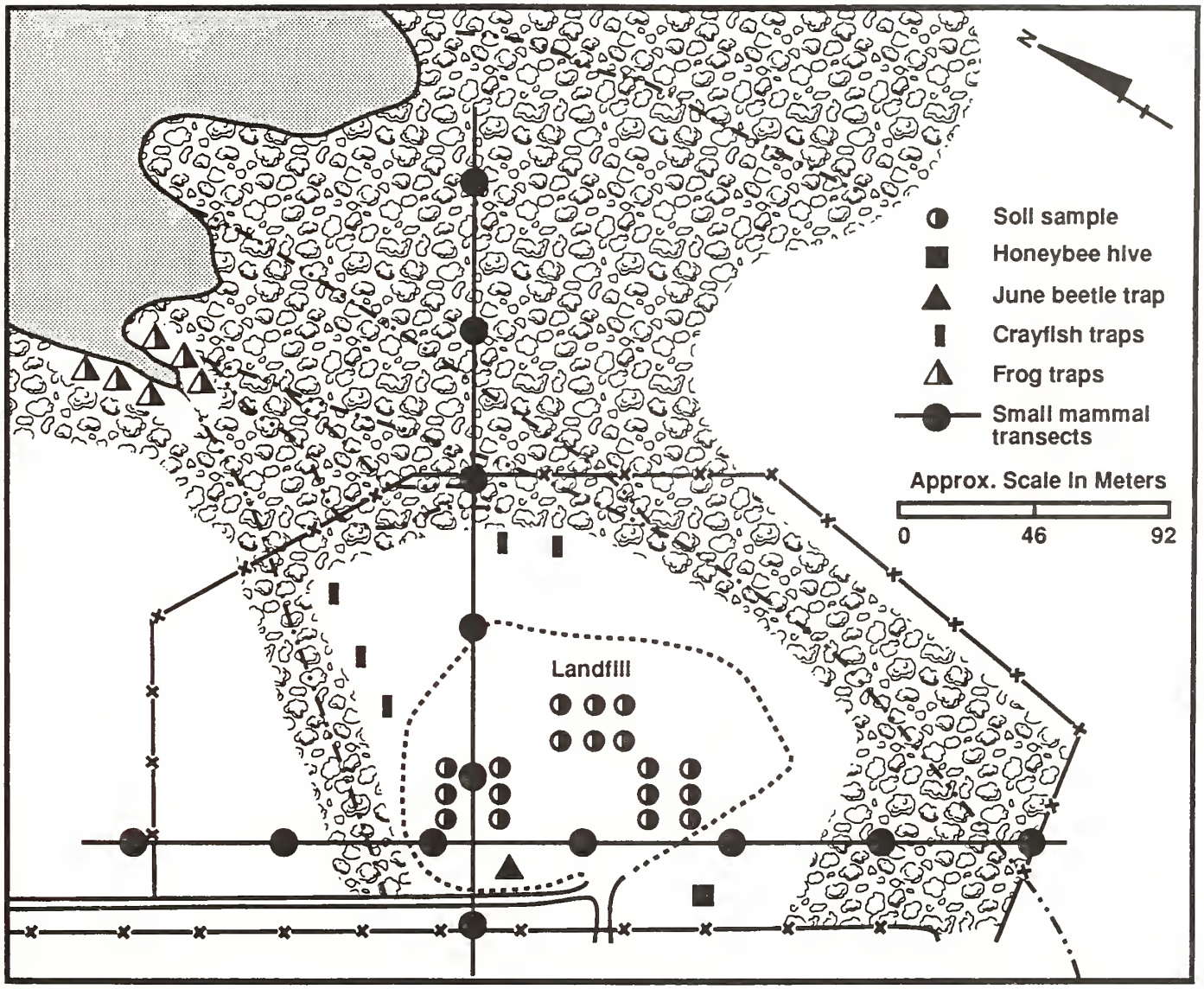

Figure 2. Location of hazardous waste, soil samples, small animal transects, June beetle traps, frog traps, crayfish samples, and honeybee hive at Area 9 Landfill.

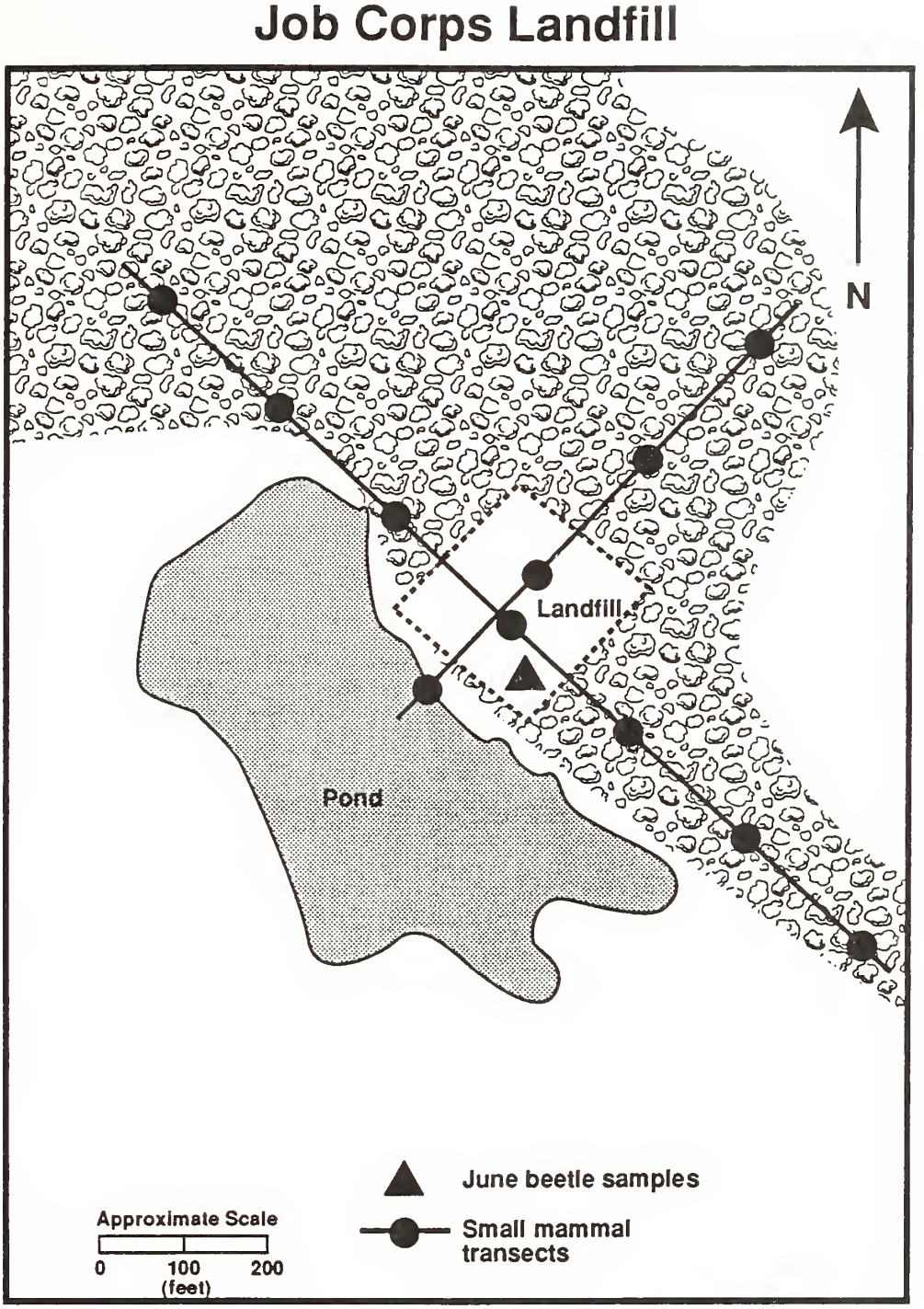

Figure 3. Location of hazardous waste landfill, small mammal transects, and June beetle traps at the Job Corps Landfill site.

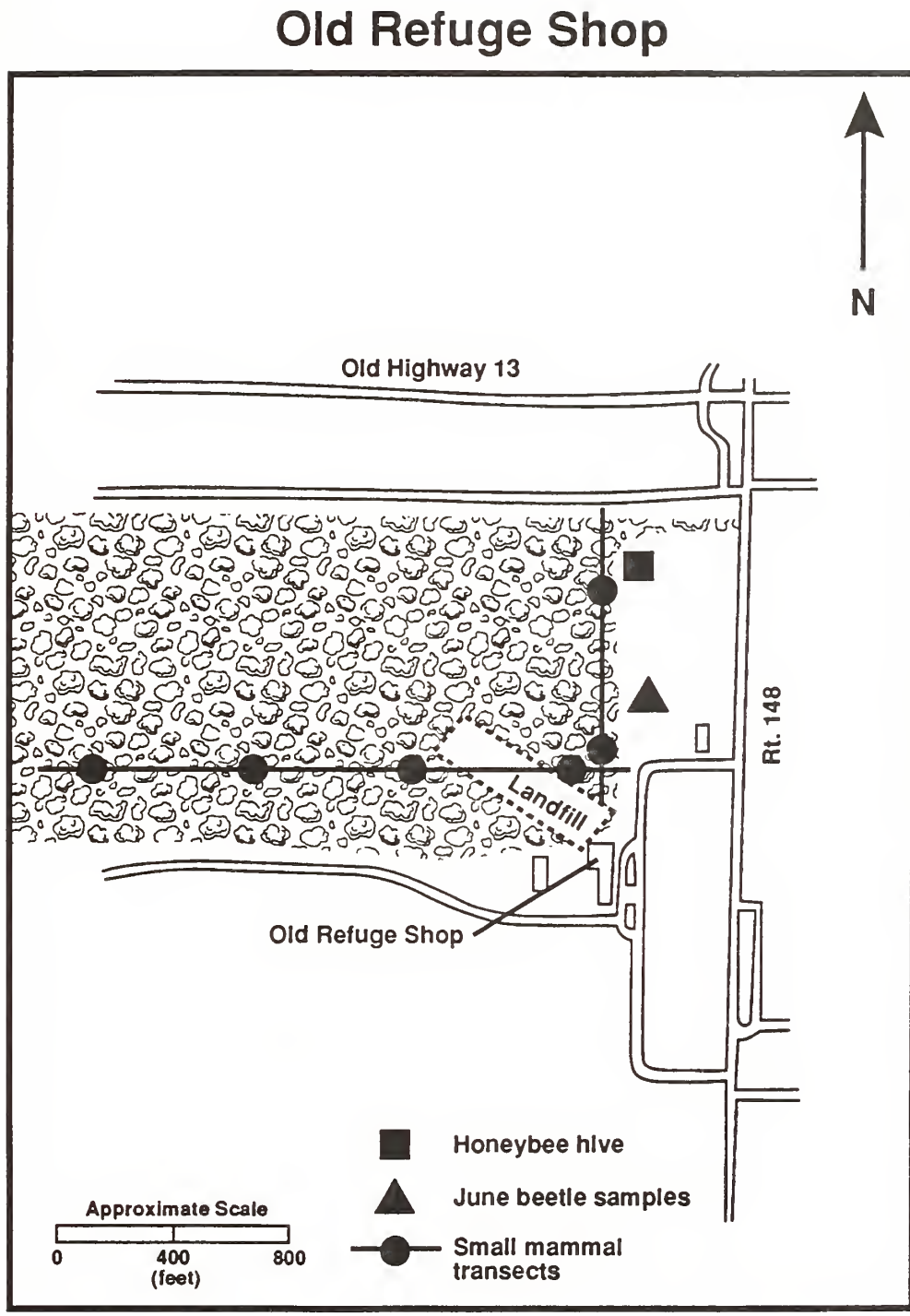

Figure 4. Location of hazardous waste landfill, small mammal transects, and June beetle traps at the Old Refuge Shop site.

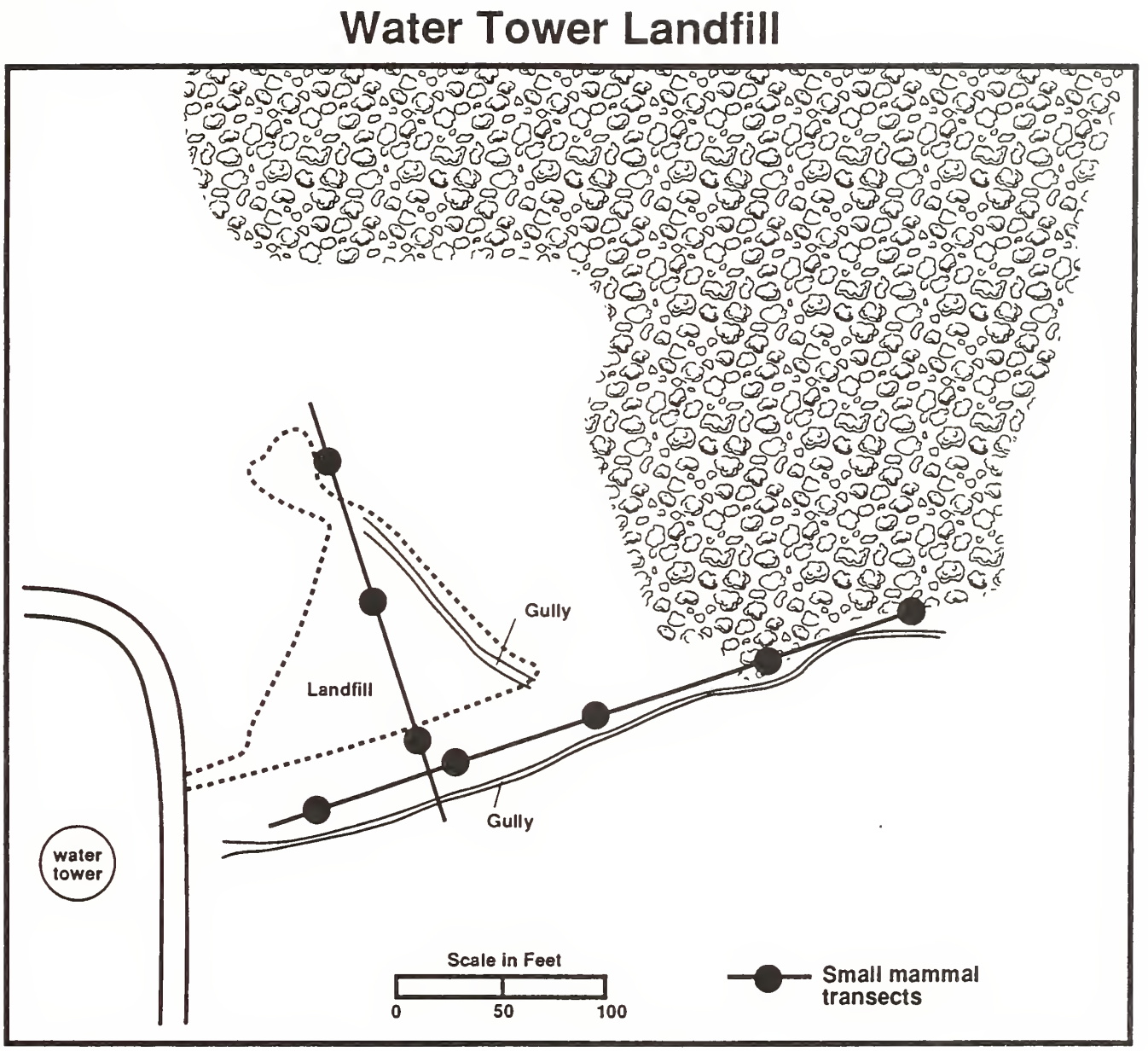

Figure 5. Location of hazardous waste site and small mammal transects at the Water Tower Landfill.

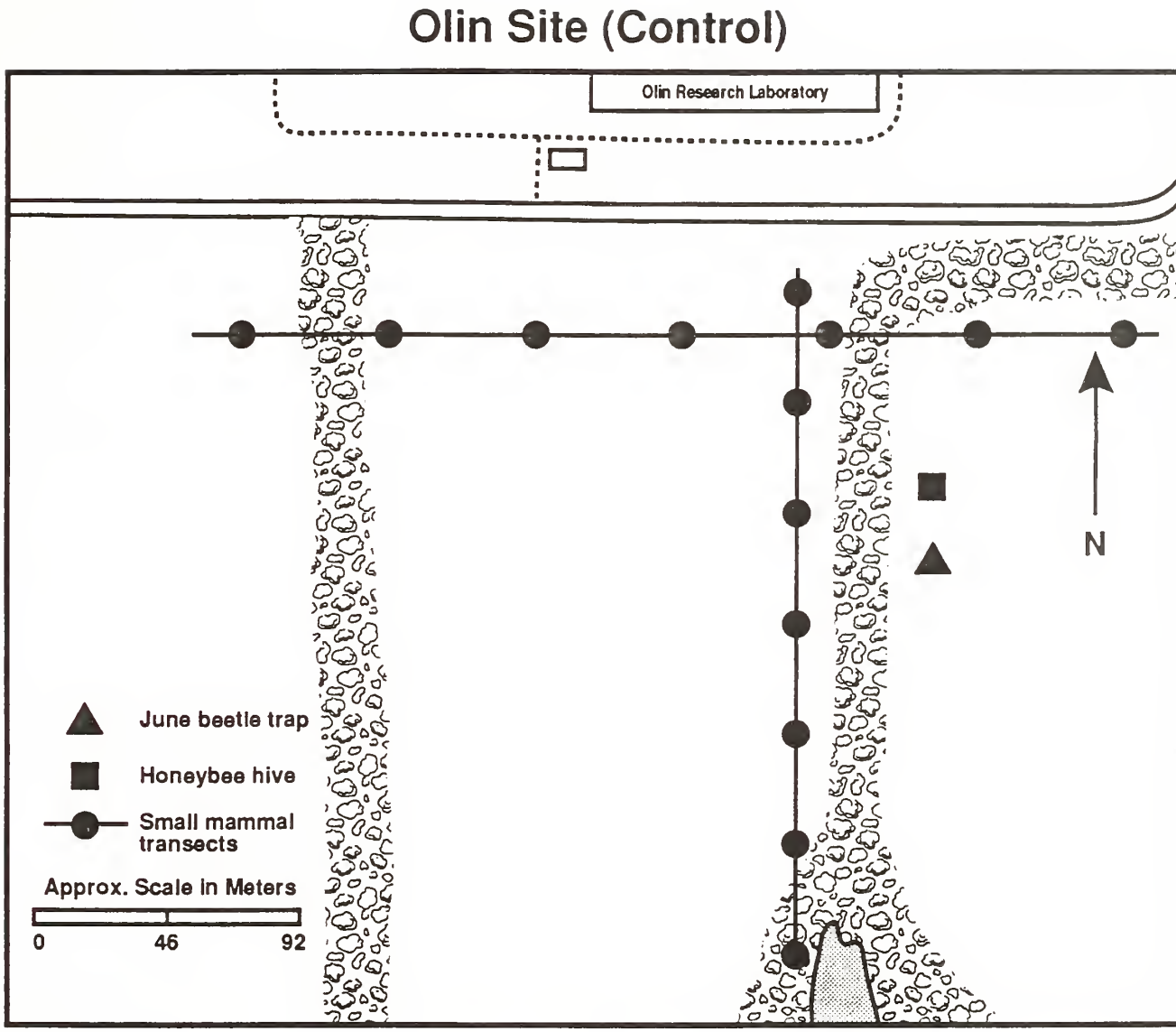

Figure 6. Location of transects, June beetle trap and honeybee hive at the Olin site (control).

APPENDIX B

Summary of insect abundance at Test Sites

Table 1. Abundance of insect families found at the hazardous waste site and control site during the 1989 season.

Order	Family	Number Observed (total)[a] HWS	Olin
Collembola	Entomobryidae	343	234
	Sminthuridae	1	1
Microcoryphia	Meinertellidae	32	0
Odonata	Zygoptera	1	0
Orthoptera	Acrididae	18	2
	Gryllacrididae	4	1
	Gryllidae	40	19
	Tettigoniidae	1	0
Blattaria	Blattellidae	9	0
	Blattidae	1	0
Hemiptera	Coreidae	3	1
	Lygaeidae	2	3
	Reduviidae	1	3
	Rhopallidae	1	0
	Tingidae	0	4
Homoptera	Aphididae	1	18
	Acanaloniidae	0	1
	Cercopidae	0	3
	Cicadellidae	20	8
Coleoptera	Buprestidae	1	0
	Byrrhida	1	0
	Carabidae	19	20
	Curculionidae	14	4
	Chrysomelidae	32	33
	Elateridae	10	0
	Meloidae	1	6
	Phalacridae	1	0
	Languriidae	1	0
	Staphylinidae	33	44
	Scarabaeidae	0	2
Diptera	Cecidomyiidae	7	1
	Chironomidae	24	2
	Lauxaniidae	1	0
	Sciaridae	2	0
	Tabanidae	1	0

Table 1 (continued). Abundance of insect families found at the hazardous waste site and control site during the 1989 season.

Order	Family	Number Observed (total)[a] HWS	Olin
Lepidoptera	Hesperiidae	4	4
	Geometridae	0	1
Hymenoptera	Apidae	6	2
	Anthophoridae	3	2
	Formicidae	492	979
	Mutillidae	2	1
	Sphecidae	3	0

[a] Total catch for 3 plots at each site over a 9 day sampling period.